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Exploring strategies to individualize treatment with aminoglycosides and co-trimoxazole for MDR Tuberculosis

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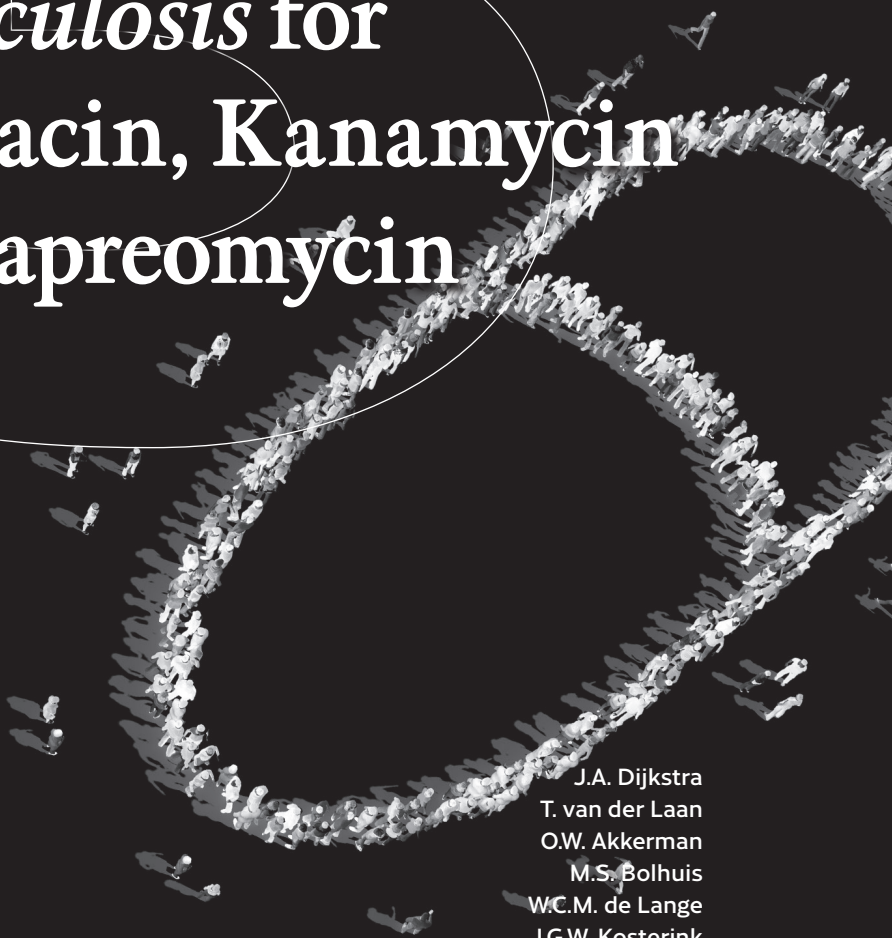
In Vitro Drug Susceptibility of *Mycobacterium Tuberculosis* for Amikacin, Kanamycin and Capreomycin



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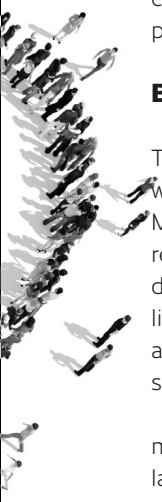
ABSTRACT

Amikacin, kanamycin and capreomycin are listed among the most important 2nd line drugs for multidrug resistant tuberculosis. Although amikacin and kanamycin are administered in the same dose and show the same pharmacokinetics they have different EUCAST breakpoints suggesting that the two drugs have a different minimal inhibitory concentrations (MIC). The aim of this paper was to investigate possible differences in MIC between the aminoglycosides and capreomycin.

Using the direct concentration method, a concentration range of amikacin, kanamycin and capreomycin (0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00, 32.00 and 64.00 mg/L) was tested against 57 clinical *Mycobacterium tuberculosis* strains. The 7H10 agar plates were examined for mycobacterial growth after 14 days.

At 2 mg/L, 48 strains (84%) were inhibited by amikacin and only five strains (9%) were inhibited by kanamycin ($p < 0.05$, Wilcoxon Signed Rank Test). The median MICs of amikacin, kanamycin and capreomycin were 2, 4 and 8 mg/L, respectively. No difference was observed between multidrug resistant and fully susceptible strains in the MIC-distribution of amikacin, kanamycin and capreomycin.

The results indicate that amikacin is more active against *M. tuberculosis* than kanamycin and capreomycin in the absolute concentration method. The impact of this difference on clinical outcome in daily practice requires a prospective study including pharmacokinetic and pharmacodynamics evaluations.



BACKGROUND

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, affects over 12 million people world wide. More than nine million new cases, and about 1.3 million deaths are documented each year.[1] Multidrug-resistant TB (MDR-TB), defined by the resistance to at least isoniazid and rifampin, requires treatment with second line drugs. MDR-TB is treated with a combination of a Group A drug (fluoroquinolones), a Group B drug (aminoglycosides and capreomycin) and other second-line agents (group C and D).[1] Aminoglycosides like amikacin and kanamycin and the glycopeptide antibiotic capreomycin can be used without a clear preference and have identical dosing schedules, according to WHO guidelines.[1]

In daily practice the drug susceptibility is usually tested using the following breakpoints: 1 mg/L for amikacin, 2.5 - 4 mg/L for kanamycin and 1.25 – 10 mg/L for capreomycin.[2,3] There is a large difference observed between the MIC in solid and liquid media, with MIC differences of 1.0 – 2.0 mg/L for amikacin, 1.25 – 5.0 mg/L for kanamycin and 1.25 – 10.0 mg/L for capreomycin, respectively.[2]

The difference in breakpoint between amikacin, kanamycin and capreomycin is supported by literature, indicating that the minimum inhibitory concentration (MIC) of amikacin in vitro is lower than the MIC of kanamycin and capreomycin. The MIC of amikacin ranged from < 0.5 – 1 mg/L in 54 clinical isolates, while the kanamycin MIC ranged from 1 – 4 mg/L in the same panel of isolates.[4] This finding of differences in MICs for the two different aminoglycosides has also been found in other studies.[5,6] This could imply that kanamycin is less effective than amikacin in vitro, since a higher concentration is needed to inhibit growth of the same strain. It is suggested that this difference in MIC may be caused by the butyric acid moiety at the R3 position of kanamycin reducing its activity against *M. tuberculosis*.[5]

This difference in MIC might be clinically relevant, since the effectiveness of aminoglycosides is likely to depend on the C_{max}/MIC ratio.[7,8] Using a pharmcokinetic/pharmacodynamic (PK/PD)

approach this therefore suggests that dosing of amikacin and kanamycin should be adjusted according to their C_{max} and MIC values to reach optimal efficacy. From an earlier PK study it is known that C_{max} does not differ between amikacin and kanamycin when given in the same dose of 15 mg/kg.[9] More recently, the chance to develop hearing loss is lower with reduced aminoglycoside dosing based on peak- and trough levels. Therapeutic drug monitoring of aminoglycosides seems promising[10] and feasible as bioanalytical immunoassays and mass spectrometry methods have been made available for amikacin as well as kanamycin.[11,12] Obviously, to reach the same C_{max}/MIC ratio for amikacin and kanamycin, having different MIC values, a difference in dosing should be employed.

Apparently, there is an inconsistency between available data according to the C_{max} and MIC of *M. tuberculosis* for amikacin and kanamycin from a PK/PD point of view and the current WHO dosing recommendations. However, there is a paucity of data on the difference in MIC between amikacin, kanamycin and capreomycin using the same panel of strains. Therefore, we tested in vitro susceptibility of amikacin, kanamycin and capreomycin against clinical non-MDR and MDR isolates of *M. tuberculosis*.

METHODS

Susceptibility testing

The absolute concentration method, we use in this study, is a widely used method to test drug susceptibility.[13] In brief, 7H10 medium with different concentrations of amikacin, kanamycin and capreomycin separately (0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00, 32.00 and 64.00 mg/L) are sterilized for 10 min at 121 °C. All compounds have shown to be stable in medium after sterilization 14. After sterilizing, the bottles are cooled down to 50 °C and oleic acid-dextrose-catalase (OADC; Becton Dickinson and Company) is added. The pH is set at 6.6 ± 0.2 after the addition of OADC. Twenty five well plates are prepared and each filled with 2.5 mL the sterilized medium containing different concentrations of the drugs, or no addition.

A small loop of bacteria are suspended in 40 mL distilled water and homogenized. The concentration of bacteria is adjusted to between 2 x 105 and 10 x 105 CFU/ml. In total, 10 µL of this suspension was added to the 25-well plates. The two control wells are inoculated with 10 µL and a 1/100 dilution of the suspension, respectively. Inhibition of > 99% of the growth is considered prevention of growth. In addition, the control *M. tuberculosis* strain: H37Rv (ATCC 27924) is tested in duplicate. The growth of the bacilli was checked after 14 days. The MIC is determined when the growth in the control wells is sufficient.

Target attainment analysis

PK/PD parameters are based on published data.[15] The volume of distribution is cal-culated for 1,000 virtual patients based on the volume of distribution mean ± standard deviation in MDR-TB patients with a normal distribution using the random number generator of SPSS version 23 (IBM, Amaronk, NY). This volume of distribution and the recommended aminoglycoside dosage of 15 mg/kg, is used to calculate the C_{max} is calculated by C_{max} = dose / volume of distribution. The attainable C_{max}/MIC is calculated based on this C_{max}. Target attainment is calculated for the classical C_{max}/MIC >10 target, and the suggested C_{max}/MIC >20 and C_{max}/MIC >70 targets.[8,16] In addition, the cumulative fraction of response (CFR) is calculated (17). The target attainment analysis is performed for both amikacin and kanamycin, since the PK profile is highly similar.[15]

This analysis is not performed for capreomycin, since the PK profile is largely unknown.

RESULTS

Susceptibility testing

In total, 57 available clinical *M. tuberculosis* strains and two control strains (ATCC 27924 (H37Rv) sensitive; ATCC 35827 kana R) were tested using the direct concentration method. The MICs measured with the H37Rv reference strain were: amikacin 2 mg/L, capreomycin 4 mg/L and kanamycin 2 mg/L. The the clinical isolates' MIC distribution of amikacin, kanamycin and capreomycin is shown in *figure 1*.

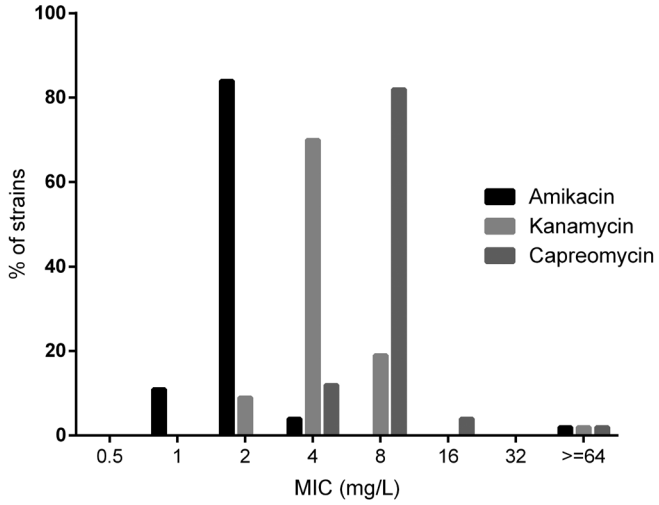


Figure 1. MIC distribution of amikacin, kanamycin and capreomycin.

At 2 mg/L, 48 strains (84%) were inhibited by amikacin and five strains (9%) by kanamycin. At 8.0 mg/L, all strains were inhibited by both aminoglycosides. The difference in MICs between amikacin and kanamycin is displayed in *table 1*. A Wilcoxon Signed Rank Test showed that the MICs significantly differed between amikacin and kanamycin ($Z = -6.6$, $p < 0.05$). The median amikacin and kanamycin MIC was 2 and 4 mg/L, respectively. The median MIC of capreomycin was 8 mg/L. The MIC of capreomycin differed significantly from the MICs of amikacin ($Z = -6.9$, $P < 0.05$) and kanamycin ($Z = -6.2$, $P < 0.05$).

Table 1. Susceptibility of *M. tuberculosis* for amikacin and kanamycin

Minimum inhibitory concentration*	Number of strains (%)
Amikacin > kanamycin	0
Kanamycin > amikacin	12 (21%)
Equal amikacin and kanamycin MICs	45 (79%)
Amikacin > Capreomycin	0
Capreomycin > Amikacin	51 (89%)
Equal amikacin and capreomycin MIC	6 (11%)
Kanamycin > Capreomycin	0
Capreomycin > Kanamycin	2 (4%)
Equal kanamycin and capreomycin MIC	55 (96%)
Total number of strains	**57**

** MICs with one or more intermediate dilution steps were considered different*

Comparing individual strains, the MIC of amikacin was more than one dilution step lower than the MIC of kanamycin in 21% of all strains. No strains were more susceptible to kanamycin than to amikacin. The capreomycin MIC was higher than the amikacin MIC in 51 (89%) of all tested strains. In the other six strains, MICs were comparable (within one dilution step difference). In two strains (4%), capreomycin MICs were more than one dilution step higher than the kanamycin MICs. All other strains showed similar MICs within one dilution step of kanamycin and capreomycin.

The MICs of amikacin, kanamycin and capreomycin did not differ between MDR-TB and non-MDR-TB strains (Mann-Whitney test, P = 0.98, 0.38, 0.74, respectively).

Probability of target attainment

The probability of target attainment is pictured in *figure 2*, based at a dosage of 15 mg/kg. The probability to achieve a C_{max}/MIC ratio of 10 at a MIC of 2 mg/L is 100% for both amikacin and kanamycin. At an MIC of 4 mg/L, the probability of target attainment is 99%. The probability to achieve target achievement with an MIC of 8 mg/L is 39%. With a target C_{max}/MIC ratio of >20, target attainment is still 99% at an MIC of 2 mg/L. Targeting a higher C_{max}/MIC ratio of >70, target attainment at an MIC of 2 mg/L falls to 6%. The CFR of each aminoglycoside at different C_{max}/MIC targets is displayed in *table 3*.

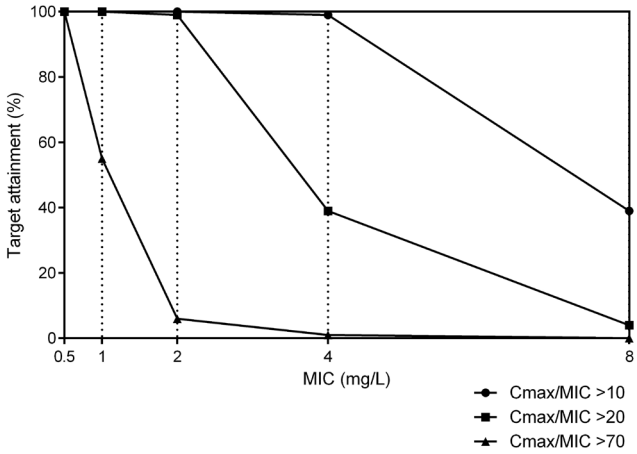


Figure 2. Target attainment analysis of amikacin and kanamycin at a dosage of 15 mg/kg at various C_{max}/MIC ratios.

Table 2. Observed MICs in other reports

First author (year)	Source strain (n)	MIC AMK (mg/L)	MIC KAN (mg/L)	Ref.
Ho et al. (1997)	Susceptible (23)	≤0.5-1	1-4	4
	Resistant to streptomycin (14)	≤0.5-1	1-2	
	MDR (10)	≤0.5-1	1-4	
	H37Rv	1	2	
Rastogi et al. (1996)	Susceptible (5)	0.5	2-4	5
	MDR (3)	0.5-1	2.0	
	INH-STR res (1)	0.5	4.0	
	H37Rv	0.5	2.0	
Sanders et al. (1982)	H37Rv	2	8	17

Table 3. Cumulative fraction of response (CFR)

	Amikacin	Kanamycin
C_{max}/MIC > 10	98%	86%
C_{max}/MIC > 20	96%	37%
C_{max}/MIC > 70	11%	1%

DISCUSSION

This is, to our knowledge, the first systematic study comparing the MIC of amikacin, kanamycin and capreomycin using a direct concentration method.

At least one dilution step in growth inhibition between drugs is required for a significant difference in MICs. We have shown that, in general, amikacin is more active in vitro in killing *M. tuberculosis* than kanamycin and capreomycin. This difference in MIC between amikacin and kanamycin is also confirmed in other reports, as shown in *table 2*. In all three reports, the MICs of amikacin are lower than those of kanamycin.[4,5,17] Based on these findings, one can conclude that amikacin is more effective than kanamycin and capreomycin in killing *M. tuberculosis* in vitro.

This difference could be caused by mutations in the eis promotor gene.[18,19] This gene is responsible for aminoglycoside low-level resistance by the production of acetyltransferase, which inactivates aminoglycosides. However, this enzyme has a larger affinity for kanamycin than for amikacin.[18,19] A mutation in the eis promotor gene could therefore result in reduced susceptibility to kanamycin, with only a minor impact on the amikacin MIC. After a mutation of the eis promotor gene, the MIC of amikacin was 0.25 – 2 mg/L (wildtype 0.25 – 0.5 mg/L), while the MICs of kanamycin were largely affected: MIC of mutants ranged from 5 – 20 mg/L (wildtype 0.6 – 2.5 mg/L).[20]

It is generally assumed that the efficacy of aminoglycosides depends on the C_{max}/MIC ratio.7 This relationship has however just recently been established for *M. tuberculosis*.[8] Based on the differences in MIC, it can be debated whether amikacin and kanamycin are equally effective at the same dose. In situations where information on both MIC and C_{max} was available and therapeutic drug monitoring was applied, preliminary results show that in the presence of other active drugs, a lower dosage of amikacin was adequate.[16]

The method used in this study has some limitations, which are related to the slow multiplication rate of mycobacteria. More modern techniques, such as the BACTEC MGIT960, use the oxygen consumption to measure growth. However, these methods have other limitations, such as a relatively low specificity for streptomycin and a low sensitivity for kanamycin resistance.[21] Furthermore, these methods are relatively expensive in comparison with the direct concentration method.

In most parts of the world, drug susceptibility testing (DST) for second line drugs is not integrated in standard care. According to a recent WHO report, isolates of 24% of all new world wide cases were subjected to DST of rifampicin, and 53% of isolates of previously treated patients were tested for drug susceptibility.[1] It is therefore important to provide information on the wild type MIC distributions to determine the optimal dosing schedule in MDR-TB treatment when DST is not available. With the Sensititre MycoTB Plate, it is also possible to determine the MIC of various anti-TB drugs against TB. This method could also be applied in low resource settings.[22]

In addition to the earlier data to identify the C_{max}/MIC target for *M. tuberculosis* it is of interest to repeat these experiments in the presence of other anti-TB drugs.[8] This information may help to tailor the dose needed to reach a sufficient C_{max}/MIC ratio that likely translates in treatment success.[16]

CONCLUSION

The MIC of amikacin appears to be slightly, but significantly lower in vitro in comparison with the MIC of kanamycin and capreomycin in clinical isolates. The impact on clinical outcome requires a prospective study including pharmacokinetic and pharmacodynamics evaluations.

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